ELSEVIER

Toxicon 47 (2006) 371–379

TOXICON

www.elsevier.com/locate/toxicon

Intoxication by *Ipomoea sericophylla* and *Ipomoea riedelii* in goats in the state of Paraíba, Northeastern Brazil

R.C. Barbosa [a], F. Riet-Correa [b,*], R.M.T. Medeiros [b], E.F. Lima [a], S.S. Barros [c], E.J. Gimeno [d], R.J. Molyneux [e], D.R. Gardner [f]

[a] *Programa de Pós-Graduação em Ciência Veterinária, Universidade Rural de Pernambuco, CEP 52171-900, Recife PE, Brazil*
[b] *Centro de Saúde e Tecnologia Rural, UFCG, Campus de Patos, 58700-000, Patos PB, Brazil*
[c] *Faculdade de Veterinária, UFPel, Campus Universitário, , 96100-000, Pelotas, RS, Brazil*
[d] *Instituto de Patologia, Universidad Nacional de La Plata, PO Box 296, 1900, La Plata, Argentina*
[e] *USDA/ARS Western Regional Research Laboratory, 800 Buchanan Street, Albany, CA 94710, USA*
[f] *USDA/ARS Poisonous Plants Research Laboratory, 1150 East 1400 North, Logan, UT 84341, USA*

Received 26 August 2005; revised 11 November 2005; accepted 16 November 2005

Abstract

A disease of the nervous system was observed in goats from two farms of the semiarid of the state of Paraíba, northeastern Brazil. *Ipomoea sericophylla* was found in one farm and *I. riedelii* in the other. Both plants were administered experimentally to five goats each. Both plants induced clinical signs similar to those observed in spontaneous cases. Two goats died spontaneously and five were euthanatized. Three goats recovered after the withdrawal of the plants. Histological examination showed that all goats that died spontaneously or were euthanized had diffuse vacuolation of neurons, macrophages of lymphatic tissues, and epithelial cells of pancreas, thyroid, renal tubules and liver. On electron microscopy of Purkinje cells, numerous dilated membrane bordered vacuoles were identified as lysosomes. On lectin-histochemical analysis, cerebellar cells gave positive reactions to *Concanavalia ensiformis*, *Triticum vulgaris*, and succinylated-*T. vulgaris*, which indicate the storage of α-D-mannose, α-D-glucose, β-D-*N*-acetyl-glucosamine, and acetyl-neuraminic acid. The chemical analysis of *I. sericophylla* and *I. riedelii* showed 0.11 and 0.14% of swainsonine, respectively. The latter also contained calystegines B_1, B_2 and C_1. It is concluded that *I. sericophylla* and *I. riedelli* cause a lysosomal storage disease.

Keywords: Storage diseases; Swainsonine; Ipomoea sericophylla; Ipomoea riedelii; Toxic plants

Acquired glycoprotein storage diseases are caused by plants of the genera *Swainsona*, *Oxytropis*, *Astragalus* (James et al., 1970; James and Panter, 1989; Dorling et al., 1978; Hartley et al., 1989), *Ipomoea* (de Balogh et al., 1999; Armién, 2000; Rodriguez Armesto et al., 2004) and *Sida carpinifolia* (Driemeier et al., 2000) which contain swainsonine a well known inhibitor of lysosomal α-mannosidase and Golgi mannosidase II (Huxtable and Dorling, 1982; Molyneux et al., 1995b; de Balogh et al., 1999; Colodel et al., 2002a; Haraguchi et al., 2003).

Ipomoea carnea Jacq. subsp. *fistulosa* (Mart.ex Choisy) D.F. Austin has been recognized as toxic since 1960 in northeastern Brazil (Tokarnia et al., 1960) affecting goats, sheep and cattle. More recently the disease was characterized as a glycoprotein storage diseases (Armién, 2000)

* Corresponding author. Tel.: +55 83 34233409; fax: +55 83 341214659.
E-mail address: franklin.riet@pesquisador.cnpq.br (F. Riet-Correa).

0041-0101/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2005.11.010

caused by the presence of swainsonine in the plant (de Balogh et al. 1999; Haraguchi et al., 2003; Medeiros et al., 2004). Another plant causing glycoprotein storage diseases in goats (Driemeier et al., 2000), sheep (Seitz et al., 2005), and horses (Loretti et al., 2003) in southern Brazil is *Sida carpinifolia*, which also contains swainsonine (Colodel et al., 2002a).

The object of this paper is to report the clinical signs, histological and ultrastructural findings, and lectin-histochemical analysis of the cerebellar cells in the spontaneous and experimental intoxication by *Ipomoea sericophylla* and *I. riedelii* in goats, as well as the determination of the active principle of these plants.

1. Material and methods

1.1. Study of the spontaneous disease

Two farms, one in the municipality of São Sebastião do Umbuzeiro, and another in the Municipality of Zabelé, state of Paraíba, Brazil where the disease was occurring were visited to determine the epidemiology of the disease and observe the clinical signs. One goat with clinical signs from the farm in the municipality of São Sebastião do Umbuzeiro was euthanized by electric shock following anesthesia with sodium pentobarbitone and necropsied. After fixation, the CNS was sectioned in 4–5 mm transverse sections. Transverse sections taken from the cervical, thoracic and lumbar spinal cord, medulla oblongata, pons, rostral colliculi, thalamus, basal nuclei and internal capsule, cortex, cerebellar peduncles, and cerebellum were examined histologically. Longitudinal sections of the spinal cord were also studied. Samples of liver, kidney, lung, lymph nodes, spleen, thyroid, adrenal, stomach, small and large intestine, cardiac, skeletal muscles and peripheral nerves were also fixed in 10% neutral formalin. All tissues were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. Selected sections of the CNS were also stained with Luxol fast blue for myelin.

Other farms in the municipalities of São Sebastião do Umbuzeiro, Zabelé and Monteiro were visited to ask the farmers about the occurrence of the disease and for observation of the paddocks.

1.2. Identification of the plant

Samples of the whole plant of the two different species of *Ipomoea* found in each farm were sent for botanical identification to the Empresa Pernambucana de Pesquisa Agropecuária, Recife, state of Pernambuco, Brazil.

1.3. Experimental reproduction of the disease

The plants used in the experiments were collected in the municipalities of São Sebastião do Umbuzeiro (*I. sericophylla*), and Zabelé (*I. riedelii*). For the experiments with fresh plants they were collected weekly and kept at 3–4 °C until administered. For the experiment with dry plants, the plants were dried in the shade, ground and mixed with concentrate ration.

Fourteen 4–12-month old Moxotó goats were used; seven (nos 1–7), to reproduce the intoxication by *I. sericophylla* and seven (nos 8–14) to reproduce the intoxication by *I. riedelii*. General information for the experimental studies is summarized in Table 1. Data on the amount of plants consumed were collected daily, and the goats were weighted at start and end of experiment.

1.3.1. Experiments with I. sericophylla

Goats 1 and 2 consumed the fresh plant as the only food for 24 and 34 days, respectively. From day 34 to day 40, goat 2 received the fresh plant ad libitum, but also commercial cencentrate ration in an amount equivalent to 1% live weight. Goats 1 and 2 were euthanized 1 day after the end of plant consumption. Goat 3 received the fresh plant ad libitum, and commercial concentrate ration in an amount equivalent to 1% live weight, during 38 days. After this period, the plant was substituted by *Cynodon dactylon* (Tifton grass) hay. After the withdrawal of the plant the animal was examined daily to observe regression of the clinical signs. Goats 4 and 5 received, daily, a mixture of 50% dry ground *I. sericophylla* and 50% cencentrate ration, in amounts equivalent to 0.8% of their live weight, which represents a daily dose of 4 g/kg bw of dry plant. They also received *C. dactylon* hay ad libitum. Goat 4 received the plant for 53 days. After this period the animal was observed for regression of the clinical signs, fed with 1% bw of concentrate ration and *C. dactylon* hay ad libitum. Goat 5 was euthanized and necropsied, 1 day after the withdrawal of the plant. The period of plant administration was determined by the intensity of clinical signs or by the disposition of the animals to ingest it. Goats 6 and 7 were used as controls, receiving cencentrate ration (1% bw) and *C. dactylon* hay ad libitum.

1.3.2. Experiments with I. riedelii

Goats 8 and 9 consumed the fresh plant as the only food for 22 days. Goat 10 received the fresh plant ad libitum during 60 days, but also commercial cencentrate ration in an amount equivalent to 1% live weight. Goats 8 and 9 died spontaneously and goat 10 was euthanized 3 days after the end of consumption. After this period the plant was substituted by *C. dactylon* hay. Goats 11 and 12 received, daily, a mixture of 50% dry ground *I. riedelii* and 50% cencentrate ration, in amounts equivalent to 0.6% of their live weight, which represents a daily dose of 3 g/kg bw of dry plant. They also received *C. dactylon* hay ad libitum. Goat 11 received the plant for 46 days. After this period the animal was observed for regression of the clinical signs, fed with 1% bw of cencentrate ration and *C. dactylon* hay ad libitum. Goat 12 received the plant for 39 days, and was

Table 1
Mean daily and total dose of *I. sericophylla* and *I. riedelii* consumed by the experimental goats, weight of the animals at the start and end of the administration, clinical signs, and outcome of the intoxication

Goat no.	Plant material	Initial weight (kg)	Final weight (kg)	Daily dose (g/kg)	Total dose (g/kg)	Ingestion period (days)	Onset of clinical signs (days)	Duration of clinical signs (days)	Euthanasia (days after the end of ingestion) or death	Recovery (days after the end of ingestion)
1	Fresh Is[a]	7	5.8	112	2688	24	22	4	1	
2	Fresh Is	9	8	59	2419	40	20	23	1	
3	Fresh Is	7.6	5.5	25	950	38	28	38[c]		4
4	Dry Is	15	12	2.3	122	53	18	36[c]		11
5	Dry Is	16.1	13.5	2.7	97	36	22	15	1	
6		8	11	0	0	0	No signs		Non-euthanized	
7		13	18	0	0	0	No signs		Non-euthanized	
8	Fresh Ir[b]	12	9.7	95.8	2107.6	22	11	12	Death	
9	Fresh Ir	9.5	8.5	115	2530	22	11	12	Death	
10	Fresh Ir	11	10.8	82.4	4944	60	No signs	3		
11	Dry Ir	8.5	8,0	3.0	138	46	26	20[c]		14
12	Dry Ir	8.5	7.7	2.8	109.2	39	18	23	1	
13		11.3	14.6	0	0	0	No signs	Non-euthanized		
14		15	16.6	0	0	0	No signs	Non-euthanized		

[a] *Ipomoea sericophylla.*
[b] *Ipomoea riedelii*.
[c] Until withdrawal of the plant.

euthanized and necropsied 1 day after the withdrawal of the plant. The period of plant administration was determined by the intensity of clinical signs or by the disposition of the animals to ingest it. Goats 13 and 14 were used as controls, receiving cencentrate ration (1% bw) and *C. dactylon* hay ad libitum.

1.3.3. Clinical, macroscopic and microscopic examinations

During the experiments the animals were examined daily, performing a detailed nervous system examination, including the head raising test and the stand up test. The first consists of raising the head of the animal for about 60 s and then suddenly releasing it to observe the presence of cerebellar signs (Pienaar et al., 1976). The stand up test consists in putting the animal in lateral recumbence then observing the time to get up, and the presence of coordination problems (Armien, 2000).

Goats 1, 2, 5, 10 and 11 were euthanized by electric shock following anesthesia with sodium pentobarbitone. These goats and goats 8, 9, and 12, which died spontaneously, were necropsied. Histological examination was performed with the methods reported for the spontaneous case.

1.3.4. Electron microscopy

Samples of the cerebellum of goats 5 and 10 were fixed in 2% glutaraldehyde with 2% paraformaldehyde, in 0.4 M cacodylate buffer (pH 7.4), post fixed in 1% osmium tetroxide buffered in 0.4 M sodium cacodylate (pH 7.4), and embedded in Epon 812. Semithin sections were stained with methylene blue. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a EM 109 Zeiss transmission electron microscope at 80 kw.

1.3.5. Lectin histochemistry

Paraffin wax-embedded sections (5 μm) from goats 1, 5, 9 and 10 were used. After dewaxing, sections were treated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature (to inhibit the endogenous peroxidase), rinsed several times in 0.01 M PBS (pH 7.2), and treated with 0.1% bovine serum albumin in PBS for 15 min. The sections were then incubated for 1 h at room temperature with biotinylated lectins. Nine lectins (Vector Laboratories, Burlingame, CA, USA) with different specificities were used, as follows: Con A (*C. ensiformis*), binding specificity α–D-Man and αD-Glc; DBA (*Dolichos biflorus*), binding specificity α-D-GalNAc; SBA (*Glycine max*), binding specificity α-D-GalNAc, α-D-GalNAc and α and β-Gal; PNA (*Arachis hypogaea*), binding specificity β-D-Gal and (1–3)Gal NAc; RCA-I (*Ricinus communis*-I), binding specificity β-D-Gal and α-D-Gal; UEA-1 (*Ulex europaeus-I*), binding specificity α-L-Fuc; WGA (*Triticum vulgaris*), binding specificity α-D-GlcNAc and NeuNAc; sWGA (Succinyl-WGA), binding to β-(1–4)D-GlcNAc; and BS-I

(*Bandeirea simplicifolia-I*), binding to α-D-Gal. The optimal lectin concentration was 30 μg/ml in PBS for all lectins, except PNA, which was applied at a concentration of 10 μg/ml. The slides were ncubated with an avidin–biotin–peroxidase complex (ABC) (vector) for 45 min. The horseradish peroxidase was activated by incubation for 1–2 min with a diaminobenzidine commercial kit (DakoCytomation, Carpinteria, CA, USA). Specimens were rinsed in distillated water, dehydrated with graded ethanol solutions, cleared in xylene and mounted in Permount (Fisher Scientific International, Inc., Liberty Lane Hampton, NH, USA). Controls for lectin staining included: exposure to horseradish-peroxidase and substrate medium without lectin; and blocking by incubation with the appropriate blocking sugars (0.1–0.2 M in PBS) for 1 h at room temperature before applying to the sections. The intensity of lectin binding was subjectively scored from 0 (none) to 3 (strongly positive). Two cerebellums of slaughtered, 1 year old, Moxotó goats were used as control.

1.4. Identification of the active principles of the plants

Swainsonine content of *I. sericophylla* and *I. riedelii* was measured by liquid chromatography-mass spectrometry using procedures previously described (Gardner et al., 2001). Duplicate samples (100 mg) of the air-dried plant material were extracted and the swainsonine isolated by ion-exchange resin. The final aqueous extract was then analyzed by liquid chromatography–mass spectrometry. The presence of swainsonine was verified using gas chromatography–mass spectrometry after a portion of the aqueous extract was dried and the residue derivatized by addition of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine to convert swainsonine to its timethylsilyl ether derivative. Calystegines were analyzed by gas chromatography–mass spectrometry by the method of Molyneux et al. (1995). Dried, ground leaves (approximately, 1 g) were extracted with methanol (MeOH) for 16 h in a Soxhlet apparatus, and the alkaloidal fraction was purified by ion-exchange chromatography on a 5×0.5 cm column of Dowex 50W-X8 (NH_4^+ form). The 0.5% aqueous ammonium hydroxide eluate was evaporated to dryness to give the base fraction, containing any alkaloids and basic amino acids. Portions of this material were used for gas chromatography–mass spectometric (GC–MS) analysis of the trimethylsilyl (TMSi) derivatives, prepared by treatment with *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) in pyridine at 60 °C for 1 h. Analyses were performed on a Hewlett–Packard 5890 Series II instrument equipped with a 5971 mass-selective detector operating at 70 eV, an on-column injector, and a 60 m×0.32 mm i.d. SE-30 fused Si column. The column was temperature programmed from 120–300° at 10°/min.

2. Results

2.1. Identification of the plant

The plant collected in the municipality of São Sebastião do Umbuzeiro was identified as *I. sericophylla* Meisn (Fig. 1). Voucher specimens kept in the herbarium Dárdano de Andrade Lima were identified as: São Sebastião do Umbuzeiro, Paraíba, N° 66099 (IPA), and São Sebastião do Umbuzeiro, Paraíba, N° 66102 (IPA). The plant collected in the municipality of Zabelé was identified as *I. riedelii* Meisn (Fig. 2). Voucher specimens were identified as Monteiro, Paraíba, N° 60345 (IPA), and Monteiro, Paraíba, N° 60344 (IPA).

2.2. Spontaneous cases

The farm in the municipality of São Sebastião do Umbuzeiro was visited in June 2001. In the farm, 17 out of 18 goats of different ages showed clinical signs characterized by rough hair coat, depression, weight loss, and nervous signs including difficulties in rising, ataxia, hypermetria, wide-based stance, intention tremors, spastic paresis mainly in the hind legs, abnormal postural reactions, nystagmus, head tilting, depressed tongue or lip tonus, and occasionally other signs of cranial nerves impairment. Clinical signs got worst when the goats were disturbed or frightened. The animals had been affected for more than 6 months. A goat with non-reversible clinical signs (Figs. 3 and 4) was euthanized. No macroscopic lesions were observed. The main histologic lesions were in the cerebellum with the loss of Purkinje cells, which had been substituted by Bergman glia (Fig. 5). Walleriana-type degeneration, due to neuronal loss was observed mainly in the cerebellar white matter. In this year, probably because the rainy season was already finished no species of *Ipomoea* were found. In the following year during the rainy season, it was observed that the paddock was severely invaded by *I. sericophylla*. The

Fig. 1. *Ipomoea sericophylla*. Meisn. Municipality of São Sebastião do Umbuzeiro, State of Paraíba, Brazil.

Fig. 2. *Ipomoea riedelii*. Meisn. Municipality of Zabelé, State of Paraíba, Brazil.

farmer reported that the goats consumed the plant in preference to other forages.

A farm in the municipality of Zabelé, which was neighboring to São Sebastião de Umbuzeiro, was visited in April 2002. The farm had 80 goats, 46 sheep, and 24 cattle. One female, 1-year-old, goat showed clinical signs similar to those previously mentioned. This goat was brought to the University of Campina Grande, and two and a half years later showed the same clinical signs. In the year 2001, two goats were affected in this farm, in 2000, six were affected, and more than 20 had been affected in 1999. Most cases had permanent clinical signs and were culled. The disease was more frequent in 1–2 years young goats. Sheep and cattle were not affected. The paddock was severely invaded by *I. riedelii*. The farmer reported that the goats developed a preference to consume the plant, which he defined as addiction.

During the rainy season of 2003, another 10 farms were visited in the three neighboring municipalities of Zabelé, São Sebastião do Umbuzeiro, and Monteiro to ask the farmers about the occurrence of the disease and for observation of the paddocks. In four farms only *I. riedelii* was found in the paddocks, and in one only *I. sericophylla* was found. In the other farms both plants were present in the paddocks in different amounts, but generally *I. riedelii* was more frequent. In one farm there was one goat with clinical signs, and in another two goats were affected. Four farmers did not observe affected animals on their farms, and the other four reported that the disease occurred in previous years.

Fig. 3. Goat intoxicated spontaneously by *I. sericophylla* showing loss of balance due to severe intention tremors after being disturbed.

Fig. 4. Goat intoxicated spontaneously by *I. sericophylla* showing ataxia.

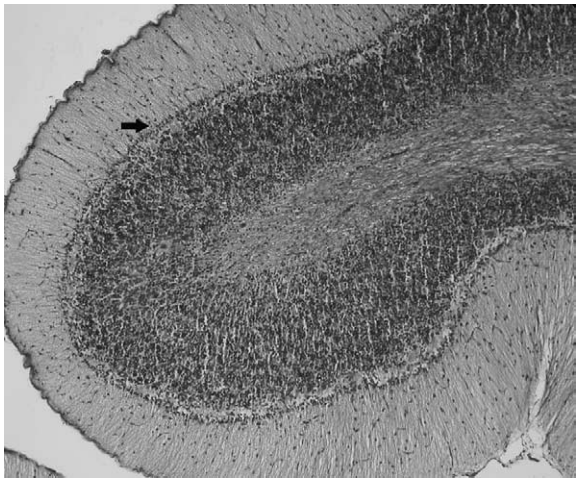

Fig. 5. Cerebellum from the goat observed in Figs. 3 and 4. There is a severe loss of Purkinje cells, which had been substituted by Bergmann glia (arrow). HE. ×10.

2.3. Experimental reproduction of the disease

2.3.1. Plant consumption, clinical signs, gross and microscopic lesions

The daily and total dose of *I. sericophylla* or *I. riedelii* consumed by the experimental goats, the weight of the animals at the start and end of the experiment, the observation of the first clinical signs, and outcome of the intoxication are presented in Table 1.

In goats 1–5, 11 and 12, first clinical signs were observed 18–28 after ingestion (Table 1). They were characterized by depression, sometimes rough hair coat, and by spending more time to rise after the stand up test. Four–7 days later, goats 2–5, 11 and 12 showed intention tremors, and loss of equilibrium and falling to the side or backward after the head raising test. Other clinical signs, observed 7–15 days after the observation of the first signs, were incoordinated, hypermetric or lateral gait, wide based standing, spastic paresis, ataxia, abnormal postural reactions, nystagmus, inappetency, rough hair coat, weight loss, and, in goat 1, lateral recumbence. Goats 8 and 9 that consumed fresh *I. riedelii* as the only food showed rough hair coat and depression on day 11 after administration, in the following days they showed weight loss and weakness; they were found dead in the morning of day 23. No clinical signs were observed in goat 10 after the ingestion of fresh *I. riedelii* for 60 days (Table 1).

Goat 4 had diarrhea on the seventh day after the start of the plant administration. Despite continuation of the plant consumption the animal recovered 6 days later. Diarrhea was again observed on day 34, but the goat recovered in 3 days. Diarrhea was also observed in goat 5 from day 6 to day 14; on day 20 it had diarrhea again, but recovered 2 days later. Goat 11 had diarrhea 19 days after the plant ingestion, recovering spontaneously in 4 days. Goat no. 5 had diarrhea and depressed food consumption after 14 days of consumption, recovering spontaneously in 2 days. The four animals continued to consume the plant during the periods that they had diarrhea.

No clinical signs were observed in the control goats 6, 7, 13 and 14.

At necropsies of goats 1, 2, 5, 10, and 12 no significant gross lesions were observed. Goats 8 and 9 had ascites, dehydration, hydropericardium, emaciation, and serous atrophy of the pericardial fat.

Histologic lesions were vacuolation of the perikaria in neurons in all regions of the nervous system, but more prominent in the Purkinje cells (Fig. 6) of the cerebellum and in neurons of the cerebellar nuclei. Axonal spheroids were observed in the granular layer of the cerebellum, cerebellar white matter, cerebellar peduncles and cerebellar nuclei (Fig. 7). Cytoplasmic vacuolation was also observed in parenchymal cells of various organs including pancreatic acinar cells, hepatocytes, epithelial cells of the kidney, follicular epithelial cells of the thyroid gland, Kupffer cells and macrophages of the lymphatic tissues. Those lesions were observed in all goats, including goat 9 that had no clinical signs.

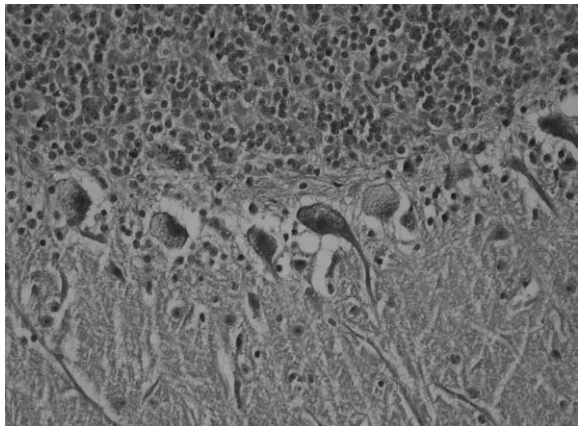

Fig. 6. Cerebellum. Sheep no. 5. There is severe vacuolation of Purkinje cells. HE. ×20.

2.3.2. Electron microscopy

The electron microscopy of the Purkinje cells showed that the vacuoles observed under light microscopy correspond to dilate lysosomes surrounded by a single layered membrane (Fig. 8). The vacuoles were empty or contained membranous fragments or small amounts of a finely granular material. The axonal spheroids in the granular layer were filled with membrane bound residual bodies and some mitochondria (Fig. 8).

2.3.3. Lectin histochemical findings

The cytoplasm of Purkinje cells, and cytoplasm of multiple cells of cerebellar granular and molecular layers of

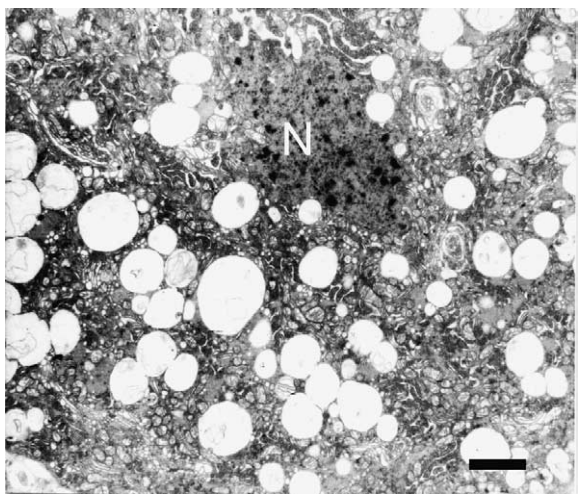

Fig. 7. Electron microscopy. Cerebellum. Purkinje cell. Numerous membrane bound vacuoles in the pericaryon, some with membrane fragments. Nucleus (N). Bar = 1 μm.

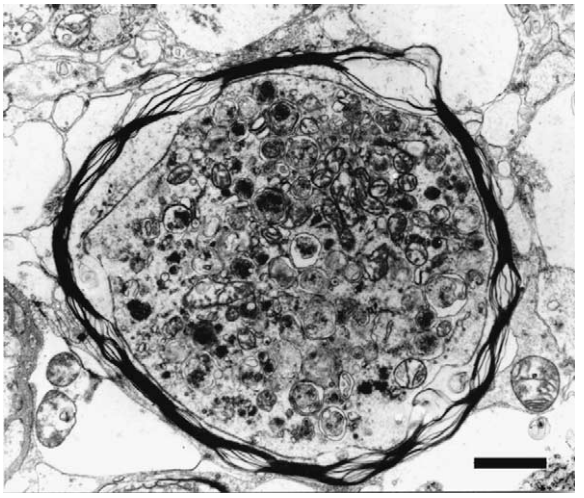

Fig. 8. Electron microscopy. Cerebellum. Granular layer. Axonal spheroid filled with membrane bound residual bodies and some mitochondria. Bar = 3 μm.

all affected goats examined (nos 1, 5, 9, and 10) stained strongly with Con A, WGA, and sWGA. These cells did not stain in corresponding slides from control animals. Lectin binding permitted the demonstration of stored material in numerous cells that appeared normal with the haematoxylin and eosin stain. Additionally, a partial weak reaction of Purkinje cells to UEA-I, PNA, RCA-I, SBA and DBA was observed in some cells from affected and control goats.

2.4. Identification of the active principle of the plants

The chemical analysis of the plants showed 0.11% swainsonine in *I. sericophylla* and 0.14% swainsonine in *I. riedelii*. Small amounts of calystegines B_1, B_2 and C_1 were also present in *I. riedelii* but no calystegines were detected in *I. sericophylla*.

3. Discussion

The results reported in this paper demonstrated that *I. riedelli* and *I. sericophylla* cause a swainsonine induced glycoprotein storage diseases.

The spontaneous disease, caused by *I. sericophylla* in the municipality of São Sebastião do Umbuzeiro, and by *I. riedelii* in the municipality of Zabelé, state of Paraíba, is also observed in other farms in the region, but in most of these farms both plants are found together. The intoxication is seasonal occurring during the rainy season, which extends from December–January to April–May when the plants are growing. A similar disease is caused in the Brazilian semiarid by *Ipomoea carnea* subsp *fistulosa* (Tokarnia et al., 1960; Armién, 2000; Riet-Correa et al., 2003), which also contains swainsonine (de Balogh et al., 1999; Armién, 2000; Haraguchi et al., 2003; Medeiros et al., 2004). *I. carnea* subsp *fistulosa* also affects cattle and sheep (Tokarnia et al., 1960). *I. riedelii* and *I. sericophylla* occur only during the raining season, but *I. carnea* subsp *fistulosa* is a plant found mainly around water fountains or partially flooded areas during the whole year, and the intoxication can occur at any time of the year.

Nervous signs observed in the intoxication by *Ipomoea* spp. are characteristic of lesions in the cerebellum and brain stem. Hindquarter ataxia and spastic paresis are due to propioceptive and upper motor neuron lesions, respectively; nystagmus, head tilting, decrease tonus of the tongue and/or lip are signs of cranial nerves impairment; and wide stance, loss of equilibrium, hypermetria and intention tremors are due to cerebellar lesions. Such clinical signs are similar to those observed in the intoxication by other swainsonine-containing plants, including *I. carnea*, *S. carpinifolia*, *Swainsona* spp., *Oxytropis* spp. and *Astragalus* spp. (James et al., 1970; James and Panter, 1989; Dorling et al., 1978; Hartley et al., 1989; de Balogh et al., 1999; Driemeier et al., 2000; Armien, 2000). In the experimental reproduction of the disease these signs were reversible in goats ingesting the plants for periods of 38–53 days, and showing clinical signs for 20–38 days (Table 1). On the other hand, many spontaneous cases had irreversible nervous signs, suggesting that the ingestion of the plant was for longer periods. In a recent experiment comparing the toxicity of *I. riedelii* and *I. sericophylla*, clinical signs became irreversible only in one goat out of four after the ingestion of *I. riedelii* containing 0.01% swainsonine for periods of 141 days, and in three goats ingesting *I. sericophylla* containing 0.05% swainsonine, during 75–127 days. Clinical signs started after 36–38 days of *I. sericophylla* ingestion and after 99–135 days of *I. riedelii* ingestion. This information is important for the control of the intoxication. If the animals are withdrawn from the area invaded by the plants after the first signs of intoxication they will recover. Also goats showing clinical signs for less than 30 days can recover. The cause of the irreversibility of the nervous signs after some period is the neuronal loss, which was observed, mainly in the cerebellum, in the goat intoxicated spontaneously by *I. sericophylla*. Because both toxic species of *Ipomoea* reported in this study only occur during the rainy season, which in the region extends from December–January to May–June is also probably that those irreversible signs are caused also by the consumption of the plant during more than one rainy season. One clinical sign not observed in the spontaneous cases is the diarrhea observed in goats 4, 5, 11 and 12 that ingested the dry plant. Because of the absence of diarrhea in the goats ingesting the fresh plant and also in the spontaneous cases, it is probably that this sign is caused by some chemical modification of the plant, during the drying process.

One probably important factor in the occurrence of the disease is the possibility of the goats developing a preference and apparent craving to graze these plants,

which was mentioned by the farmers. Everist (1981) reported that sheep develop a morbid appetite for *Swainsona* spp. and would eat nothing else when the plant is available. This morbid appetite, sometimes reported as addiction, had been reported also in the intoxication by other swainsonine-containing plants, including *Swainsona* spp. (Huxtable and Dorling, 1982), locoweeds (Ralphs et al., 1990, 1991), *I. carnea* (Tokarnia et al., 1960; de Balogh et al., 1999; Riet-Correa et al., 2003) and *S. carpinifolia* (Colodel et al., 2002b). In animals ingesting locoweeds it is suggested that this behavior is due to feed preference and habituation, not to a true addiction (Ralphs et al., 1990, 1991). Because of the potential development of a preference to graze *Ipomoea* spp. it should be recommended that affected goats not be allowed to return to areas where these plants occur after the clinical recuperation. It is very probable also that goats that consume *Ipomoea* spp. containing swainsonine induce other goats to ingest the plant by social facilitation, as occurs with locoweeds (Ralphs et al., 1994).

The pattern of lectin staining observed in Purkinje cells partially agrees with the results reported for locoweed, swainsonine, *S. carpinifolia* and *Ipomoea* spp. toxicosis and for mannosidosis in humans, cats, and calves (Alroy et al., 1985; de Balogh et al., 1999; Driemeier et al., 2000; Armien, 2000; Rodriguez Armesto et al., 2004). The reaction was clear to sWGA and WGA which indicates the expression of β-D-*N*-acetyl-glucosaminase and acetyl-neuraminic acid, and Con-A, specific for αDmannose and αDglucose (Goldstein and Hayes, 1978; Goldstein et al., 1980). A weak partial reaction was observed on Purkinje cells to UEA-I, PNA, RCA-I, SBA and DBA in affected and control tissues. WGA and RCA-I binding have been found in normal rat and chicken cerebellums (Damjanov, 1987) and Con-A, DBA, UEA-I and WGA binding occurs in the embryonic cerebellum of the chicken (Viejo Tirado et al., 1994).

The presence of 0.14 and 0.11% (dry matter) of swainsonine in *I. riedelii* and *I. sericophylla*, respectively, demonstrated that the disease is due to inhibition of lysosomal α-mannosidase and Golgi mannosidase II by this substance. This swainsonine concentration is high when compared with the swainsonine content of dry *S. carpinifolia* and dry *Astragalus lentiginosus* which contain 0.006% (Colodel et al., 2002b), and 0.007% (Molyneux and James, 1982), respectively. It is also higher than the levels found in fresh leaves of *I. carnea* (0.0029%) (Haraguchi et al., 2003). A threshold for toxicity is difficult to establish for swainsonine, but a conservative approach suggests that levels in excess than 0.001% of the dry matter should be of concern (Molyneux et al., 1995a). In other samples of *I riedelii* and *I. sericophylla*, collected in 2003, the concentration of swainsonine was 0.01 and 0.05%, respectively, demonstrating the variations in swainsonine concentration of the same plants in different years. Both plants collected in 2003 were also toxic experimentally: *I. sericophylla* at doses 2 g of dry plant per kg bw; and *I. riedelii* at 4 g/kg bw (Barbosa et al., unpublished data).

Calystegins B_1, B_2 and C_1 were found in the samples of *I. riedelii* analyzed in 2002, but not in the samples analyzed in 2003. The toxic effects of calystegines to animals had not been determined, but they are strong inhibitors of glycosidases, mainly β-glucosidase, and α-galactosidase. Recently, the occurrence of ataxia in cattle was associated with the presence of different calystegines in *Ipomoea* spp (Dorling et al., 2004).

Acknowledgements

This work was financially supported by the Programa de Apoio a Núcleos de Excelência (PRONEX-Grant N° 001/04, CNPq, FAPESQ, MCT).

References

Alroy, J., Orgad, U., Ucci, A.A., Gavris, V.E., 1985. Swainsonine toxicosis mimics lectin histochemistry of mannosidosis. Vet. Pathol. 22, 311–315.

Armién, A.G., 2000. Vergeichende klinische und morphologische Undersuchungen zur spontanen und experimentellen Vergiftung durch Ipomoea fistulosa(Convulvulaceae) bei Ziegen. Inagural-Dissertation Erlangung des Doctorgrades beim Fachbereich Veterinärmedizin der Justus-Liebig Universität GieBen, 212p.

Colodel, E.M., Gardner, D.R., Zlotowski, P., Driemeier, D., 2002a. Identification of swainsonine as a glycoside inhibitor responsible for *Sida carpinifolia* poisoning. Vet. Hum. Toxicol. 44, 177–178.

Colodel, E.M., Driemeier, D., Loretti, A.P., Gimeno, E.J., Traverso, S.D., Seitz, A.L., Zlotowski, P., 2002b. Aspectos clínicos e patológicos da intoxicação por *Sida carpinilolia* (Malvaceae) em caprinos no Rio Grande do Sul. Pesq. Vet. Bras. 22, 51–57.

Damjanov, I., 1987. Lectin citochemistry and histochemistry. Lab. Invest. 57, 5–20.

de Balogh, K.K.I.M., Dimande, A.P., Van Der Lugt, J.J., Molyneux, R.J., Naude, T.W., Welman, W.G., 1999. A lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. J. Vet. Diag. Invest. 11, 266–273.

Dorling, P.R., Huxtable, C.R., Vogel, P., 1978. Lysosomal storage in *Swainsona* spp. toxicosis: an induced mannosidosis. Neuropathol. Appl. Neurobiol. 4, 285–291.

Dorling, P.R., Colegate, S.M., Allen, J.G., Nickels, R., Mitchell, A.A., Main, D.C., Madin, B., 2004. Calystegines isolated from *Ipomoea*spp. possible associated with an ataxia syndrome in cattle in North Western Australia. In: Acamovic, T., Stewart, C.S., Pennycott, T.W. (Eds.), Poisonous Plants and Related Toxins. CABI Publishing, Wallingford, UK, pp. 140–145.

Driemeier, D., Colodel, E.M., Gimeno, J.E., Barros, S.S., 2000. Lysosomal storage disease caused by *Sida carpinifolia* poisoning in goats. Vet. Pathol. 37, 153–159.

Everist, S.L., 1981. Poisonous Plants of Australia, second ed. Angus & Robertson, Sydney Australia, 966 p.

Gardner, D.R., Molyneux, R.J., Ralphs, M.H., 2001. Analysis of swainsonine: extraction methods, detection and measurement in populations of locoweeds (*Oxytropis* spp.). J. Agric. Food Chem. 49, 4573–4580.

Goldstein, I.J., Hayes, C.E., 1978. The lectins: carbohydrate-binding proteins of plant and animals. Adv. Carbohydr. Chem. Biochem. 35, 127–340.

Goldstein, I.J., Hayes, C.E., Monsigny, M., Osawa, T., Sharon, N., 1980. What should be called a lectin ? Nature 285, 66.

Haraguchi, M., Gorniak, S.L., Ikeda, K., Minami, H., Kato, A., Watson, A.A., Nash, R., Molyneux, R.J., Asano, N., 2003. Alkaloidal components in the poisonous plant, *Ipomoea carnea* (Convolvulaceae). J. Agric. Food Chem. 51, 4995–5000.

Hartley, W.J., Baker, D.C., James, L.F., 1989. Comparative pathological aspects of locoweed and *Swainsona* poisoning of livestock. In: James, L.F., Elbein, A.D., Molyneux, R.J., Warren, C.D. (Eds.), Swainsonine and Related Glycosidase Inhibitors. Iowa State University Press, Ames, IA, pp. 50–56.

Huxtable, C.R., Dorling, P.R., 1982. Poisoning of livestock by *Swainsona* spp.: current status. Aust. Vet. J. 59, 50–53.

James, L.F., Panter, K.E., 1989. Locoweed poisoning in livestock. In: James, L.F., Elbein, A.D., Molyneux, R.J., Warren, C.D. (Eds.), Swainsonine and Related Glycosidase Inhibitors. Iowa State University Press, Ames, pp. 23–38.

James, L.F., van Kempen, K.R., Harley, W.J., 1970. Comparative pathology of *Astragalus* (locoweed) and *Swainsona* poisoning in sheep. Vet. Pathol. 7, 116–125.

Loretti, A.P., Colodel, E.M., Gimeno, E.J., Driemeier, D., 2003. Lysosomal storage disease in *Sida carpinifolia* toxicosis: an induced mannosidosis in horses. Equine Vet. J. 35, 434–438.

Medeiros, R.M.T., Riet-Correa, F., Barbosa, R.C., Lima, E.F., Neto, S.A.G., Tabosa, I., 2004. Plants toxic for domestic animals in the state of Paraíba, Northeastern Brazi. In: Acamovic, T., Stewart, C.S., Pennycott, T.W. (Eds.), Poisonings Plants and Related Toxins. CABI Publishing, Glasgow, pp. 151–156.

Molyneux, R.J., James, L.F., Ralphs, M.H., Pfister, J.A., Panter, K.P., Nash, R.J., 1995a. Polyhydroxy alkaloids glycosidase inhibitors from poisonous plants of global distribution: analysis and identification. In: Colegate, S.M., Dorling, P.R. (Eds.), Plant-Associated Toxins—Agricultural, Phitochemical and Ecological Aspects. CAB International, Wallingford, UK, pp. 107–112.

Molyneux, R.J., James, L.F., 1982. Loco intoxication: indolizidine alkaloid of spotted locoweed (*Astragalus lentiginosus*). Science 216, 190–191.

Molyneux, R.J., McKenzie, R.A., O'Sullivan, B.M., Elbein, A.D., 1995b. Identification of the glycosidase inhibitors swainsonine and calystegine B_2 in weir vine (*Ipomoea* sp. Q6 aff. *calobra*) and correlation with toxicity. J. Nat. Prod. 6, 878–886.

Pienaar, J.G., Kellerman, T.S., Basson, P.A., Jenkins, W.L., Vahrmeijer, J., 1976. Maldronksiekte in cattle. A neuronopathy caused by *Solanum kwebense*. Onderstepoort J. Vet. Res. 43, 67–74.

Ralphs, M.H., Panter, K.E., James, L.F., 1990. Feed preferences and habituation of sheep poisoned by locoweed. J. Anim. Sci. 68, 1354–1362.

Ralphs, M.H., Panter, K.E., James, L.F., 1991. Grazing behavior and forage preference of sheep with chronic locoweed toxicosis suggest no addiction. J. Range Manage. 44, 208–209.

Ralphs, M.H., Graham, D., James, L.F., 1994. Social facilitation influences cattle to graze locoweeds. J. Range Manage. 47, 123–126.

Riet-Correa, F., Tabosa, I.M., Azevedo, E.O., Medeiros, R.M., Simões, S.V.D., Dantas, A.F., Alves, C.J., Nobre, V.M.T., Athayde, A.C., Gomes, A.A., Lima, E.F., 2003. Doenças dos ruminantes e eqüinos no semi-árido da Paraíba. Semi-Árido Em Foco, Patos 1, 2–86.

Rodriguez Armesto, R., Repetto, A.E., Ortega, H.H., Peralta, C.J., Pensiero, J.F., Salvetti, N.R., 2004. Intoxicación en cabras por ingestión de *Ipomoea hieronymi* var. *calchaquina* en la Provincia de Catamarca, Argentina. Veterinaria Argentina 21, 332–341.

Seitz, A.L., Colodel, E.M., Schmitz, M., Gimeno, E.J., Driemeier, D., 2005. *Sida carpinifolia* (Malvaceae) poisoning in sheep. Vet. Rec. 156, 386–388.

Tokarnia, C.H., Dobereiner, J., Canella, C.F.C., 1960. Estudo experimental sobre a toxidez do 'canudo' (*Ipomoea fistulosa* Mart.) em runinantes. Arq. Biol. An., Rio de Janeiro 3, 59–71.

Viejo Tirado, F., Peña Melián, A., Puerta Fonollá, J., 1994. Lectin-binding patterns in the development of the cerebellum. Anat. Embryol. (Berl.) 189, 169–179.